one revolution, the β returns to the ATP waiting state again; however, it has not been identified where the bound Pi is released, although it was suggested to occur at +200° or +320° from the ATP-binding angle. In this study, we observed the rotations of the hybrid F_1-ATPase, $\alpha_3\beta(WT)_2\beta(E190D)_1\gamma$ with the high-speed camera. At +320° from the ATP binding angle of the incorporated mutant β(E190D), the clear pause of ~7msec was observed as reported previously (Ariga et al. Nat. Struct. Mol. Biol.). When high concentrations of Pi was added to the solution, the time constant of the new reaction was specifically prolonged upon addition of Pi, suggesting that Pi was released at +320°. Other lines of experiments also support this result.

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PTEN Inhibition Study by Synthetic 3-Deoxy-PI Derivatives

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PTEN is a tumor suppressor mutated in a large variety of human tumor cells. It antagonizes the PI3K signaling pathway by dephosphorylating the $PI(3,4,5)P_3$ at the 3 position of the inositol ring and plays an important role in cell growth, proliferation, survival and motility. 3-Deoxy-PI derivatives have cytotoxic activity against various human cancer cell lines by a mechanism thought to involve reduced Akt1 phosphorylation. However, these molecules could act as inhibitors of PTEN. A series of D-3-deoxy-PI derivatives and their enantiomers (3-deoxy-diC_8PI, 3,5-dideoxy-diC_8PI and diC_8PI) had been synthesized and studied as inhibitors of PTEN catalyzed hydrolysis of PI(3)P substrates. With diC_8PI(3)P as the substrate, the L- enantiomers are usually not as good inhibitors as the D-compounds, although there is an increase in potency with increasing deoxygenation. The short-chain lipids exist in the assay solution as a mix of mostly monomers but with some small micelles. By using D-diC_{16}PI(3)P as the substrates, the effect of the aggregation state of the substrates was also checked. When the substrates are presented to PTEN in micelles with TX-100, none of the 3-deoxy-PI derivatives are good inhibitors. However, when the substrates are presented in large unilamellar vesicles, the inhibitory behavior of the 3-deoxy-PI derivatives is similar to what was observed in the diC_8PI(3)P monomer/micelle system. The binding behavior of PTEN to PI vesicles in the presence of the deoxy-PI analogs has also been studied using FRET. The results showed that for the L-series, deoxygenation on the inositol head group increases the potency of enhancing the protein binding to the vesicles. In this case, L-3,5-dideoxy-diC_8PI can enhance binding by 50% at the lower concentration, while L-diC_8PI can only enhance binding at the high concentration larger than 1mM. These results are used to assess lipid binding sites in PTEN.

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The role of Kallikrein-kinin System in the Immune Response of Nasal Papilloma and Adventitious Sinusitis

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The work was dedicated to study the possible interconnection between the enzymatic activity of kallikrein-kinin system and the activation of immune response of benign nasal papilloma and adventitious sinusitis. 64 patients with nasal papilloma and adventitious sinusitis have been examined for the enzymatic activity of kallikrein-kinin system and for the cellular and humoral immune responses, as well as 20 healthy. The enzymatic activity of kallikrein-kinin system and antibody response were investigated by using the sera or plasma samples. Cellular immune response was evaluated by analyzing of T- lymphocytes, B- lymphocytes and O- lymphocytes. Comparative analysis of kallikrein activity and cellular immune response showed that elevation of kallikrein activity was well correlated with activation of T- lymphocytes (r=0.896) and T-supressors (r=0.975), indicating that the activity of kallikrein may be important in modulation of T-cell response. In contrast, activation of kallikrein-kinin system was not associated with induction of B-lymphocytes (r=0.578), T-helpers (r=0.694) and O-lymphocytes (r=0.569). Analysis of kallikrein activity and humoral immune response in the same study group showed that induction of kallikrein-kinin system was associated with substantial elevation of IgA (r=0.785), in contrast to lower level of IgG and IgM expression.
It is also revealed that there is a high correlation between kininase activity and the indices of B-lymphocytes (r=0.768), T-supressors (r=0.754) and concentration of IgA (r=0.889), IgG (r=0.995) and IgM (r=0.889), in it the kininase ferment plays an important part for the formation of IgG.
Our data suggested that the kallikrein-kinin system may play a regulatory role in cellular and humoral immune response and such interconnection between these two systems could be used as additional criteria for the evaluation of immune status.

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Induction Of Functional Hypoxia-inducible Factor-1α And Angiogenesis By Derivatives Of 8-hydroxyquinoline

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Hypoxia-inducible factor-1 (HIF-1) as a complex of α and β subunits mediates a ubiquitous pathway by which mammalian cells sense and respond to hypoxia. In mammalian cells, the levels and activity of HIF-1α are regulated by its post-translational hydroxylation as catalyzed HIF hydroxylases, whose inhibition is thus attractive from the perspective of developing pharmaceuticals that activate the HIF pathway and induce a pro-angiogenic response. We found that 8-hydroxyquinoline and its derivatives inhibit hydroxylation of proline and asparagine of HIF-1α with varying degrees. In addition, they completely block ubiquitination of HIF-1α, which leads to its accumulation and activation of HIF-1-mediated vascular endothelial growth factor transcription and reporter gene activity. Furthermore, in vivo organ models based on the chick chorioallantoic membrane assay demonstrate promotion of new blood vessel formation. Therefore, our results indicate that CQ analogs possess a pro-angiogenesis potential and thus might have the therapeutic utility in the treatment of ischemic diseases.

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The Exit of the Tunnel: Yeast Alcohol Dehydrogenase from the Acceptor's Point of View

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Alcohol dehydrogenase (ADH) is a popular model used to study quantum mechanical phenomena in enzyme-catalyzed reactions. Studies of α-secondary kinetic isotope effects (2° KIEs) have shown that the oxidation of benzyl alcohol by NAD^+ occurs by quantum tunneling with coupled motion of the primary and secondary hydrogens. In order to learn more about the nature of that coupling, we have measured α-secondary KIEs in the reverse reaction, i.e., for the yeast ADH (yADH) catalyzed reduction of benzaldehyde to benzyl alcohol. Preliminary results show that whether ^1H or ^2H is being transferred, the reaction maintains normal 2° KIEs ($k_H/k_T > 1$). This is most significant given that the equilibrium isotope effect for this process is inverse (EIE = 0.75). Semi-classical theory predicts an inverse 2° KIE ($k_H/k_T < 1$) for this reaction, thus the findings support a role for quantum mechanical H-tunneling in the reduction of aldehydes by yADH. Furthermore, these 2° KIEs violate the rule of the geometric mean: the semi-classical formulation of KIEs that predicts no difference in 2° KIEs upon isotopic substitution at the primary position. In this reaction, however, the magnitude of the 2° KIE decreases significantly when the transferred isotope is ^2H. Together, these results provide strong support for the model of tunneling and 1°-2° coupled motion used to describe enzymatic H-transfers.

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Mechanism of action of cyclophilin A explored by metadynamics simulations

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Peptidyl prolyl isomerases (PPiases) are ubiquitous enzymes that catalyze the interconversion of the *cis* and *trans* isomers of the peptidyl prolyl bond. Their action is crucial in several biological processes as, for instance, in cellular signalling and in the onset of several diseases. In the HIV-1 capside protein (CA), such process takes place in the uncoating and recruitment of the virion and is catalyzed by cyclophilin A (CypA). Previous studies identified several residues that play an important role in the *trans/cis* interconversion process. However, the role of some active site residues remains still obscure, since their catalytic importance depend crucially on the stabilization of both ground and transition states. Here, we report the results of classical AMBER99 calculations on a substrate fragment of the capside protein (the /HAGPIA/ peptide) in aqueous solution and in complex with CypA. By applying replica exchange metadynamics, we calculate the free energy profile of the isomerization process in both cases as a function of several reaction coordinates. We find that CypA catalyzes only one isomerization pathway in the *trans*-to-*cis* direction and enhances the stability of a particular *cis* conformer. Based on our computational results, we propose a novel hypothesis for the working mechanism of cyclophilin A that explains, for the first time, all the available data and awaits further experimental tests.